AGAROSE COMPOSITION, AQUEOUS GEL AND METHOD OF MAKING SAME

This invention relates to a finely-divided solid blend of purified agarose with a gum free from charged groups and which forms a high viscosity solution in boiling water without gelling, the blend being soluble in water to form a gel having no electroendosmosis (−Mr), and to the aqueous gel and to the method of using it as a medium for conducting isoelectric focusing.

It has previously been proposed to employ aqueous gels made from agarose as a medium in which to carry out isoelectric focusing. However, in order to achieve satisfactory isoelectric focusing, it is essential that the gel medium have extremely low electroendosmosis properties, as close to zero as possible. Despite many efforts to purify agarose by removal of the ionic or charged groups (such as sulfate and/or carboxylate) which cause electroendosmosis in such a gel, it has not been possible as a practical matter to remove all of the groups causing electroendosmosis, as a result of which even gels made from highly purified agarose display appreciable electroendosmosis values (−Mr), of the order of 0.02 or more. The magnitude of this value, although slight compared to the usual value for unpurified agarose, is sufficiently high to preclude successful widespread use of agarose gels as a medium for isoelectric focusing. It has consequently been attempted to reduce the electroendosmosis of agarose gels still further by mixing with the agarose a variety of water-soluble materials supposedly free from ionic groups, such as sucrose (Quast, J. Chromat., Vol. 54, page 405–412, 1971), polyethylene oxide (M.W. 4,000,000) or polyacrylamide (Johansson et al., Anal. Biochem., Vol. 59, pages 200–213, 1974), and methyl cellulose (Weise et al., Progress in Isoelectric Focusing and Isotachophoresis, Ed. Righetti, North-Holland Publishing Co., 1975, pages 93–98). However, the first material, sucrose has little or no effect in reducing the electroendosmosis value of agarose gel. The addition of commercially available polyacrylamide, on the other hand, has an adverse effect on gels of highly purified agarose since it itself displays measurable electroendosmosis in aqueous gel form and since it tends to hydrolyze at various pH levels, leading to the formation of carboxylate groups and a still further increase in electroendosmosis. So far as polyethylene oxide (M.W. 4,000,000) and methyl cellulose are concerned, aqueous solutions of these materials gel upon heating to temperatures approaching the boiling point; whereas agarose dissolves in water only at elevated temperatures, preferably at the boiling point. A blend of either of these two dry solid materials with agarose consequently cannot be dissolved in water, and it is possible to prepare a solution containing both agarose and one of the other materials only by dissolving them separately with careful control of temperature.

It has also been proposed in Renn et al. U.S. Pat. No. 3,527,712 to prepare dry solid agarose in rehydratable form by incorporating in it a certain kind of macromolecular hydrocolloid; neither the agarose nor the hydrocolloid is required to have any specified extent of purity, and many of the hydrocolloids disclosed as suitable contain charged or ionic groups which produce electroendosmosis. Two of the hydrocolloids disclosed are low molecular weight polyethylene oxides; 10% by weight solutions of these in water have viscosities far below 10 cps. at 25° C.; and they are ineffective in reducing the electroendosmosis value of agarose. Although guar gum, a non-ionic material, is disclosed as a hydrocolloid, neither clarified guar gum nor clarified locust bean gum which are needed for use in the present invention, are mentioned. Unclarified guar gum and unclarified locust bean gum contain hull fragments and other impurities which obscure or interefere with staining, used in isoelectric focusing procedures.

It has now been found that a dry solid blend, preferably in finely-divided form, of (1) purified agarose having an electroendosmosis (−Mr) value no greater than 0.10 with (2) a water-soluble gum free from hull fragments, free from ionic substituent i.e. charged groups, soluble by itself in water without gelling at temperatures up to and including the boiling point (100° C. at 760 mm. (Hg)) to form a viscous solution having a viscosity at a concentration no greater than 10% by weight of at least 10 cps. at 25° C., is useful in making aqueous gels having greatly reduced or no measurable electroendosmosis value over a wide range of pH values. Such gels are of great value for use as a medium in which to carry out isoelectric focusing of proteins.

The agarose employed in the blend can be any which has been sufficiently purified so that it exhibits an electroendosmosis value (−Mr) of 0.10 or less, several of which are now commercially available. Agarose having this degree of purity differs from agarose previously generally available in that such purified products display no increase in gel strength when in admixture with locust bean gum or clarified locust bean gum, whereas agarose of a lesser degree of purity (having a −Mr value greater than 0.10) does exhibit such an increase in gel strength, as described for example in Baker U.S. Pat. No. 2,466,146. The electroendosmosis value of the impure agarose cannot be eliminated or decreased to zero by blending with it a water-soluble high viscosity gum as can be that of the purified agarose.

The electroendosmosis value of the agarose is measured by preparing a 1% by weight solution of the agarose in 0.05 M pH 8.6 barbital buffer. Three milliliters of the solution is poured on a clean microscope slide and allowed to gel at room temperature. Using a squared off No. 13 needle attached to a hypodermic syringe, a single hole is aspirated from the center of the gel. A standard test solution is prepared which consists of 10 mg/ml Dextran 500 (Pharmacia) and 2 mg/ml crystalline (4×) human albumin in 0.05 M pH 8.6 barbital buffer. Using a small bore dropper, sufficient solution is added to nearly fill the aspirated hole. These slides are then placed in position for electrophoresis using paper wicks. A potential of 10 volts/cm (75 volts) is applied using constant voltage settings.

Electrophoresis is continued for three hours, then the slides removed. Visualization is accomplished in two stages. The slides are first place in denatured (3A) ethanol for 15 minutes after which time the position of the dextran can be measured with respect to the origin (center to center). After measuring, the slides are transferred to protein staining solution prepared from 0.5 g amido black in 50 ml glacial acetic acid, then made up to 500 ml with ethanol. After 15 minutes the slides are washed in a 1:1 HoAc (5%):EtOH solution to remove excess stain. An hour is sufficient although the albumin position can usually be determined after 15 minutes. The distance from the center of the spot to the center of the origin is measured.

Diagramatically this can be represented as: